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Mechanistic Aspects of Ingested Chlorine Dioxide on Thyroid Function: Impact of Oxidants on Iodide Metabolism

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Toxicological studies dealing with recent findings of health effects of drinking water disinfectants are reviewed. Experiments with monkeys and rodents indicate that the biological activity of ingested disinfectants is expressed via their chemical interaction with the mucosal epithelia, secretory products, and nutritional contents of the alimentary tract. Evidence exists that a principal partner of this redox interaction is the iodide of nutritional origin that is ubiquitous in the gastrointestinal tract. Thus the observation that subchronic exposure to chlorine dioxide (ClO_2) in drinking water decreases serum thyroxine levels in mammalian species can be best explained with changes produced in the chemical form of the bioavailable iodide. Ongoing and previously reported mechanistic studies indicate that oxidizing agents such as chlorine-based disinfectants oxidize the basal iodide content of the gastrointestinal tract. The resulting reactive iodine species readily attaches to organic matter by covalent bonding. Evidence suggests that the extent to which such iodinated organics are formed is proportional to the magnitude of the electromotive force and stoichiometry of the redox couple between iodide and the disinfectant. Because the extent of thyroid uptake of the bioavailable iodide does not decrease during ClO_2 ingestion, it seems that ClO_2 does not cause iodide deficiency of sufficient magnitude to account for the decrease in hormonogenesis. Absorption of one or more of iodinated molecules, e.g., nutrients, hormones, or cellular constituents of the alimentary tract having thyromimetic or thyroid inhibitory properties, is a better hypothesis for the effects seen.

Introduction

The inhibition of thyroxine (T_4) synthesis in monkeys (*Cercopithecus aethiops*) during subchronic exposure to chlorine dioxide (ClO_2) in drinking water (*1*) was a serendipitous and the only significant finding during the investigation of the so-called oxidative stress caused by ClO_2. Investigators involved with disinfection research (*2,3*) proposed this syndrome to explain methemoglobinemic hemolytic anemia associated with exposure to large doses of disinfectants. According to this hypothesis, disinfectants, when absorbed into the blood stream, deplete red cell glutathione, allowing ferrohemoglobin to be oxidized to ferrihemoglobin (*4,5*).

The morphologic and chemical onset of heme oxidation and erythrocyte membrane damage caused by chlorite *in vitro* (*6,7*) as well as hematologic changes in chickens and rats exposed to up to 1000 mg/L of ClO_2 *ad libitum* in drinking water were demonstrated (*8*). We were unable to elicit *in vivo* hematologic changes in monkeys using ClO_2, since *ad libitum* exposure to this disinfectant above 200 mg/L caused severe taste aversion and dehydration.

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The most surprising observation in our studies was that ClO_2 is a relatively potent thyroid inhibitor, showing clear physiologic effects at about 9 mg/kg/day dose in 11 of 13 animals studied (*1*). In this study we also showed that, in monkeys intubated with a gastric tube, ClO_2 does not survive the organic environment of the stomach, and over 98% of the oxidizing capacity of an instilled ClO_2 solution (60 ppm) disappears within a few minutes. In addition, we showed spectroscopically that mixing monkey saliva with ClO_2 solution at various reactant ratios results in the instantaneous reduction of ClO_2. Thus, neither the intact molecule nor chlorite (ClO_2^-) or chlorate (ClO_3^-) is absorbed to any significant degree from the stomach when ClO_2 is consumed.

These products of reduction and hydrolysis of ClO_2, ClO_2^-, and ClO_3^- had no observable effect on the thyroid even at much greater doses (~ 40 mg/kg/day). This observation negated the possibility that such chlorine oxide anions, at the doses used, blocked iodide uptake into the thyroid follicles. Although this pharmacologic property of another chlorine oxide, perchlorate (ClO_4^-), is a recognized therapeutic effect, it can be elicited only with doses high enough to saturate the iodine-concentrating mechanism of the thyroid gland. In contrast to ClO_2, neither hypochlorite (OCl^-) nor monochloramine

(NH_2Cl) had any effect on the monkey thyroid function in our investigation.

Later studies have proved that the thyroid inhibitory effects of ClO_2 are not limited to nonhuman primates. ClO_2 exposure elicited thyroid inhibition in neonatal rats, both by direct gavage of the pups and by exposing lactating dams to aqueous ClO_2 (*9*). Ongoing research in our laboratory has also demonstrated that the rate of decrease in serum thyroxine of rats, related to aging, was accelerated when the animals were exposed to ClO_2-treated water. In these studies we have also shown that the *in vivo* radioactive iodide uptake (RAIU) in monkeys doubles after an 8-week exposure to 100 ppm ClO_2 (*10*). Since iodine is an element that has a well-defined role in maintaining basal metabolic balance through its requirement for thyroxine synthesis, we needed to examine the interaction between disinfectants and the iodide content of the alimentary tract.

Methods and Materials

Scanning Electron Microscopy of Rat Tongues

Tongues from rats maintained on 0, 100, and 200 ppm ClO_2 solution for 8 weeks were removed at sacrifice. The tissues were trimmed, preserved in 10% buffered formalin and dehydrated by using successive steps of alcohol baths. The tissue was then dried with a SAM-DRI-780A critical point dryer (Tousimis Res. Corp., Rockville, MD), mounted, and gold-coated using a PE-5000 Sputter Coater (International Scientific Instrument Co., Mountain View, CA). Photomicrographs ($\times 100$) of the rostral region of the dorsal tongue surface were taken at a constant distance from the tip using the ETEC Autoscan Scanning Electron Microscope (Perkin Elmer Co., Hayward, CA).

Iodination of Nutrients

Nutritional biochemicals were purchased from the Sigma Chemical Company (St. Louis, MO). Complex nutrients were prepared from biological samples. Carrier-free [^{125}I] as NaI was purchased from the New England Nuclear Company (Boston, MA). Saliva and gastric juice were obtained from Rhesus monkeys under mild anesthesia by intubation. Prefilled 0.8 cm $\times$ 4 cm AG1-X8(Cl^-) anion-exchange columns were purchased from the Bio-Rad Company (Richmond, CA). This resin quantitatively traps inorganic iodide and allows total recovery of covalently bound iodine by elution with 8 N acetic acid.

Tyrosine was used as a reference test compound to establish optimal reactant ratios. To 1 mL of 0.02 N hydrochloric acid (HCl) was added 0.1 mL of 0.1 M potassium iodide (KI), containing 9×10^5 cpm [$^{125}I^-$], and 0.3 mL of 600 ppm disinfectant in a capped conical polystyrene centrifuge tube. To this mixture was added either 2 mL of 0.01 M simple nutrient molecule dissolved in aqueous buffers (pH 10.4), 2 mL of 10 mg/mL complex nutrient solution, or 100 µL of saliva or gastric juice. After mixing, the reaction was allowed to proceed at room temperature for 10 min and was stopped by the addition of 0.2 mL of 0.1 M sodium thiosulfate ($Na_2S_2O_3$). Distilled water instead of HCl was used in Chloramine (NH_2Cl) reactions. The final reaction mixture was transferred to an anion-exchange column and eluted with four to eight 1-mL aliquots of 8 N acetic acid (CH_3COOH), until the last aliquot was free of radio activity. The specific activity of the eluates was determined by gamma counting, and the extent of organification was calculated as percentage of iodide eluted from the columns.

Table 1. *In vitro* evidence of iodination of nutrients, digestive fluids, and gastric mucosa by drinking water disinfectants.

	% I^- bound in the presence of		
	ClO_2	HOCl	NH_2Cl
Simple nutrients and vitamins			
Tyrosine	51.1	22.4	7.6
4-Aminobenzoic acid (PABA)	12.8	0.2	1.0
β-Sitosterol(PABA)	11.7	0.0	0.0
Prostaglandin $F_{1\alpha}$	10.8	—	—
Arachidonic acid	9.2	2.9	1.4
Folic acid	5.0	0.8	1.7
Pyridoxal	4.2	0.0	0.0
Thioctic acid	2.7	0.0	1.4
Cholesterol water soluble	2.1	0.1	0.1
Cholecalciferol	2.1	0.9	0.5
Retinoic acid	1.5	1.2	0.3
Biotin	0.8	0.0	0.1
Pyridoxamine	0.7	0.0	0.0
Vitamin K_1	0.6	0.0	0.1
Histidine	0.4	0.0	0.2
Pyridoxine	0.2	0.0	0.0
Uridine	0.2	0.0	0.0
Cytidine	0.2	0.0	0.0
Cholic acid, Na salt	0.1	0.1	0.0
Tryptophan	0.1	0.0	0.3
Glutamic acid	0.1	0.0	0.2
Complex nutritional mixtures			
Gastric juice (monkey)	30.6[b]	—	—
	2.2[c]	0.2	0.5
Saliva (monkey)	30.2[b]	—	—
	2.1[c]	0.0	5.3
Polyoxyethylene (20)- sorbitan oleate (Tween-80)	26.4	1.1	0.2
Globulin (bovine)	12.5	0.2	8.8
Hemoglobin (human)	4.3	0.4	4.1
Monkey chow[d]			
30 ppm ClO_2	16.7		
5 ppm ClO_2	6.0		
0.5 ppm ClO_2	1.8		
Meat extract (peptone)	3.4	1.2	5.6
RNA (Calf thymus)	3.2	0.0	1.6
Corn oil (Mazola)	3.0	0.4	0.1
DNA (calf thymus)	2.4	1.3	1.3
β-Lactoglobulin (bovine milk)	0.4	0.0	0.5
Isolated stomach [d]			
15 ppm ClO_2	2.0		
5 ppm ClO_2	0.3		

[a] Hydrochloric acid was not used in the mixture.
[b] 1.0 of undiluted secretion was used.
[c] Performed on 0.1 mL fluid diluted to 2 mL.
[d] Experimental details see ref. 11

Table 2. *In vivo* evidence of iodination of feed and alimentary mucosa by drinking water disinfectants.[a]

Iodination target	Disinfectant	Effect	Reference
In vivo iodinated gastric contents	ClO_2	After ingestion of $^{131}I^-$ followed by ClO_2, the esophagus and ileum of rats contained significantly increased ($p < 0.001$) radioiodide. At 1 hr post-treatment the iodide mainly resided in the small intestine ($p < 0.05$) where it increased by 3 hr ($p < 0.001$) and disappeared by 6 hr. The stomach and colon did not contain significantly elevated amounts of iodine. The radioiodine activity of the esophagus was significantly increased ($p < 0.05$) throughout the observation period. Blood and thyroid iodine activities were not affected.	(*11*)
In vitro iodinated feed		24 hr after peroral administration significant increase of radioiodine in the ileum ($p < 0.05$) and colon ($p < 0.01$), in 24-hr feces, and decrease in urine iodide ($p < 0.001$) were seen.	
Fecal particles and nonabsorbed solutes		During 10 days *ad lib.* consumption of radioiodide with feed and exposure to 100 ppm ClO_2 via drinking water, increase in the dietary iodide loss via the feces ($p < 0.05$) was seen. A significant increase in the iodide covalently bound to fecal particles ($p < 0.001$), as well as in the iodide covalently bound to water soluble residues of feces ($p < 0.001$) was observed.	(*16*)
Organs and gastrointestinal contents		Significant increase ($p < 0.01$) in the esophagus, stomach and intestinal contents, no differences in blood, thyroid, tongue, and colon.	
Organs and gastrointestinal contents	Cl_2 100 ppm	A pattern similar in fecal clearance and organ distribution similar to that caused by ClO_2 was observed.	(*16*)
Organs and gastrointestinal contents	NH_2Cl 100 ppm	No increase above controls in fecal excretion or binding to organics were observed.	(*16*)

[a] All experiments employed carrier free ^{125}I; the observations represent covalent binding of the basal iodide naturally present in the gastrointestinal tract or feed.

Results

Table 1 summarizes the *in vitro* binding of iodine to feed, gastric mucosa, and various nutrients. Table 2 describes the significant findings of *in vivo* studies with iodine distribution and fecal clearance of covalently bound iodide in rats. Figure 1*a* is the scanning electron micrograph of the tongue of a rat receiving distilled water. Figures 1*b* and 1*c* show the tongue surfaces of rats exposed to 100 and 200 ppm ClO_2 for 8 weeks, respectively. Histology of the lower alimentary tract was negative.

Discussion

From the electron micrographs (Fig. 1) and from the observations described in Tables 1 and 2, it is apparent that ingestion of disinfectant affects primarily the mucosal surfaces of the alimentary tract and the chemical composition of nutrients within (*11*). The importance of the basal iodide content of the digestive secretions and its modification by disinfectants, as well as its possible implication in altering thyroid function, should be rationalized in terms of the biological role of the monovalent anions of the group VII elements:

Fluoride (F^-) is essential for mineralizing bone matrix and dental enamel. It is not present in body fluids in significant quantities. Fluoride is toxic when present in body fluids in detectable quantities.

Chloride (Cl^-) is the ubiquitous essential anion for electrolyte balance and is present in body fluids in decimolar quantities. It is secreted by the gastric mucosa as free acid in excess of physiologic concentrations.

Bromide (Br^-) is a toxic and xenobiotic anion. It is not normally present in body fluids.

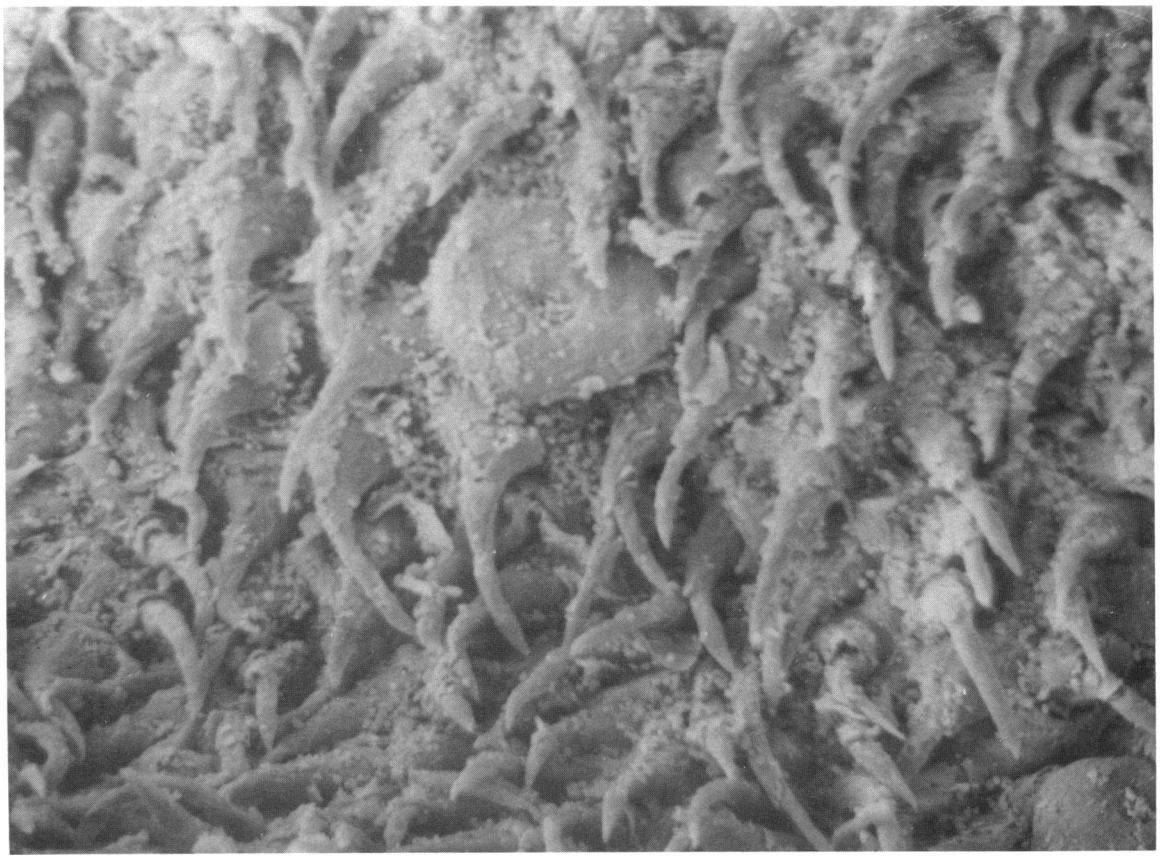
Iodide (I^-) is essential for thyroid synthesis and basal metabolism. It is concentrated in thyroid gland, salivary glands, and parietal cells. Iodide is present in body fluids in μM quantities. It is secreted in saliva and gastric juices and inhibits thyroid function at higher than required doses.

Astatite (At^-) is a rare and unstable anion of no biological significance.

In body fluids therefore, only two halides, Cl^- and I^-, must be considered as natural monovalent anions of bioessential significance and, therefore, as suitable targets for oxidation by solutions of disinfectants. Of the redox couples, the I^-/ClO_2 has the highest electromotive force computed for pure aqueous solutions (Table 3). ClO_2 also has the greatest stoichiometric capacity to oxidize I^- to a reactive I (rI) species, e.g.:

$$ClO_2 + 5I^- + 4H^+ \rightarrow Cl^- + 5\,rI + 2H_2O$$

Although the nature of rI is not known (it may be $I^\cdot$ radical, elemental I_2 iodonium cation $[I^+]$, hypoiodate $[OI^-]$, or some other active forms), it is certain that after formation, rI undergoes rapid covalent binding to organics possessing functional groups suitable for iodination. Such reactions are very likely to occur in a diversity of ways (*12*): iodination of olefinic double bonds prevalent in polyunsaturated fatty acids, triglycerides, cholesterol, and vitamins, e.g., retinol; formation of io-



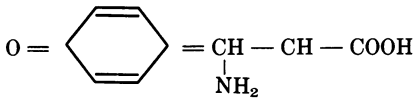
a

FIGURE 1. *In vivo* effect of ClO_2 in drinking water on the filiform papillae of the rostral tongue of the rat: (*a*) The tongue of a control animal. The filiform papillae are well organized and robust. The gustatory button and surrounding furrow is well visible in the center (×100). (*b*) 100 ppm ClO_2 exposure for 8 wks. Thinning and disorganization of the papillae is evident (×100). (*c*) 200 ppm ClO_2 exposure for 8 wks. Thinning and "beaten down" appearance of the papillae is pronounced. Histology showed attenuation of the germinal stratum and keratinization of the papillar epithelium (×100). Figure continued next page.

dolactones from unsaturated fatty acids, e.g., arachidonic acid; iodine substitution of activated aromatic and heterocyclic rings abundant in proteins in the form of tyrosine and histidine residues, vitamins, etc.; iodine substitution of activated hydrogens, such as α-methylene (CH_2) groups in ketones and aldehydes; iodohydrin formation with olefinic molecules.

According to this hypothesis, the intra-alimentary oxidation and covalent binding of I^- must be a predominant sequel to ingestion of disinfectants. The predominance of this process in a complex organic mixture is strongly supported by the example of radioiodination of antigens and antibodies, a widely and routinely practiced technique. In such reactions, radioiodide is oxidized by relatively strong agents (e.g., chloramine-T or hydrogen peroxide) in the presence of sensitive proteins (e.g., immunoglobulins, peptide hormones etc.), then rI is bound to tyrosine of the protein without disturbing the tertiary structure (e.g., the antigenicity or hapten specificity) of the protein.

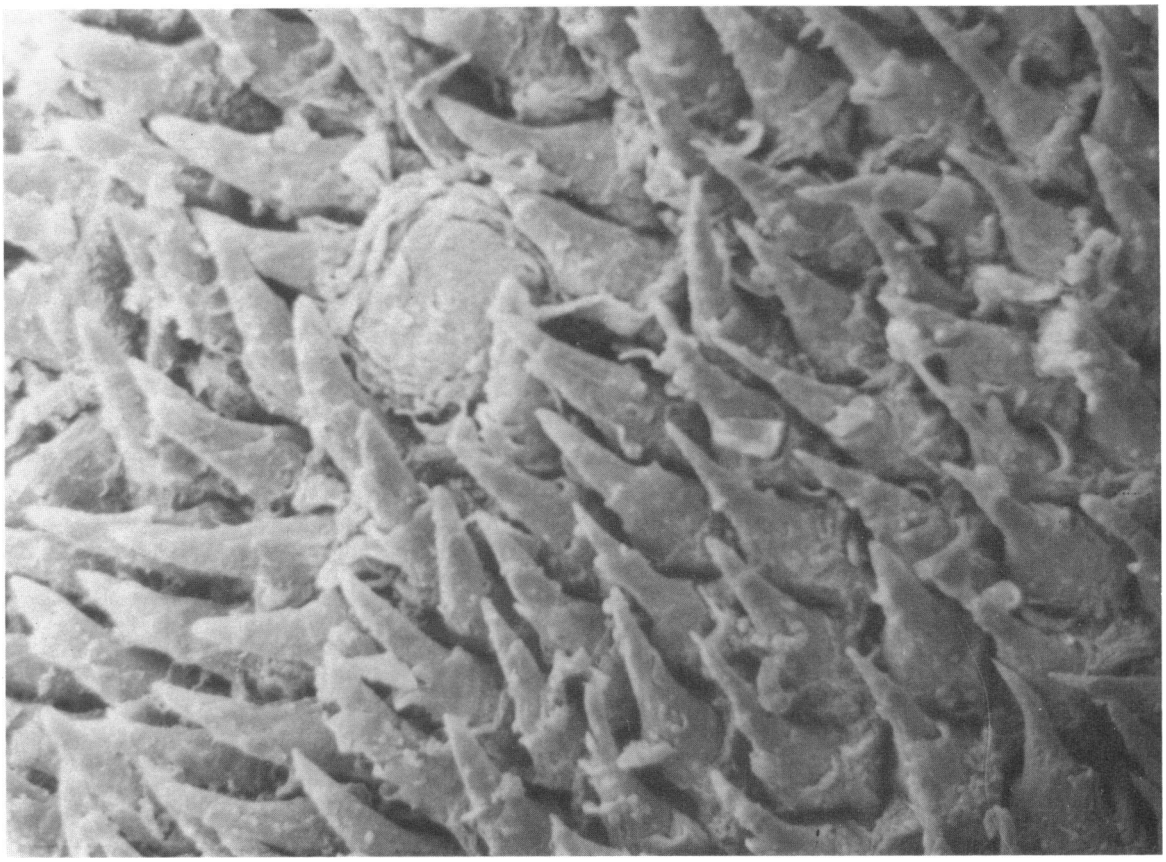
We found additional evidence that the oxidation of iodide by ClO_2 takes precedence over oxidation of organic matter and have shown that the well-known quinoidal chromogen



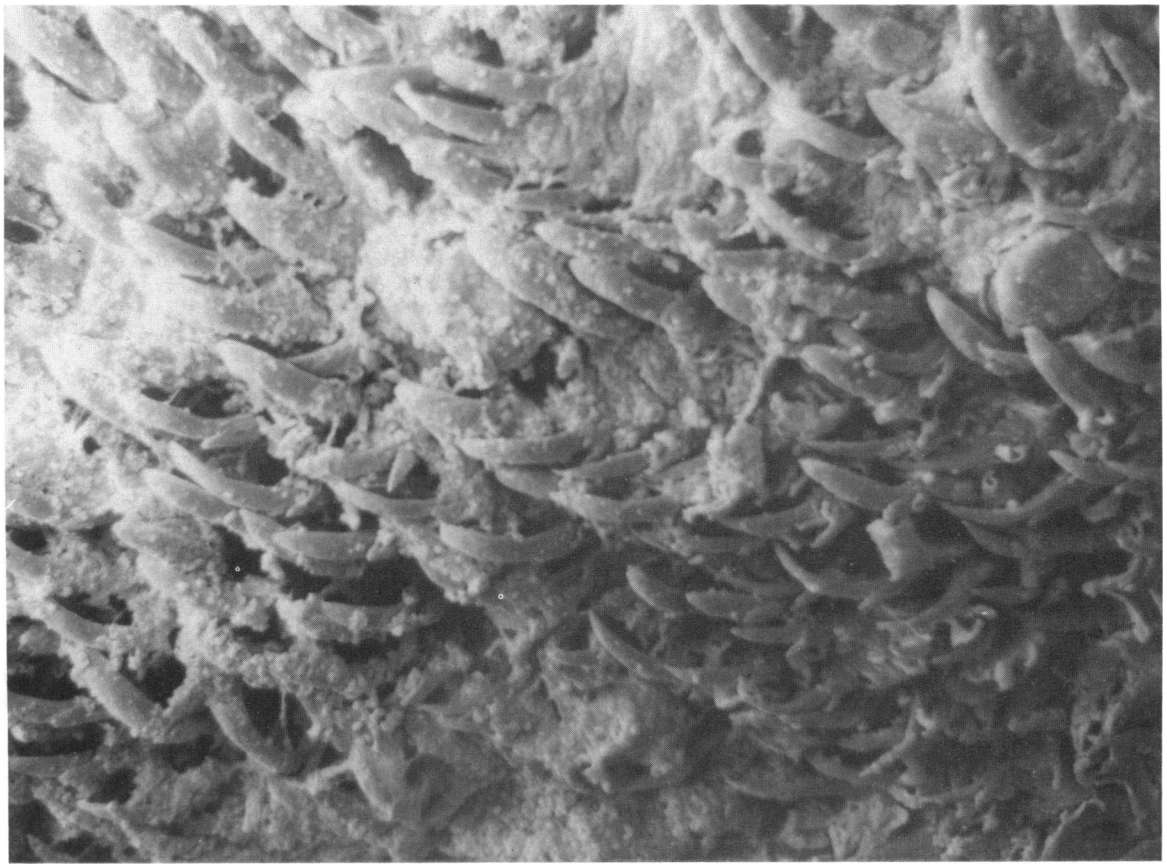
(λ_{max} = 496 nm), cannot form from tyrosine in the presence of excess iodide; instead mono- and diiodotyrosines are generated (*15*).

Results of *in vitro* iodination studies with nutrients, animal feed and isolated rat stomachs (Table 1) closely agree with the above hypothesis. Noteworthy is the clearly superior iodinating power of ClO_2 and the fact that NH_2Cl in many instances appears to be a more effective iodinating agent than HOCl. This latter observation is in contradiction with the electromotive forces (EMF) of Table 3; however, it may be explainable by the ability of hypochlorous acid (HOCl) to undergo chlorination reactions competing with the iodination process.

The *in vivo* studies with radioiodine clearly prove that intra-alimentary iodination does occur during ingestion



b



c

Table 3. Electrode potentials, equilibrium constants, and electromotive forces for redox couples between iodide and drinking water disinfectants.[a]

	Redox couple					
Products ←	Halide	Products	← Disinfectant	E, V	log K_{eq}[b]	EMF, V[c]
$2.5I_2$	$5I^- - 5e^-$	$Cl^- + 2H_2O$	$ClO_2 + 5e^- + 4H^+$	− 0.94	79.7	− 1.07
I_2	$2I^- - 2e^-$	$Cl^- + H_2O$	$HOCl + 2e^- + H^+$	− 0.95	32.3	− 1.01
I_2	$2I^- - 2e^-$	$Cl^- + OH^-$	$OCl^- + H_2O$	− 0.36	12.3	− 0.42
I_2	$2I^- - 2e^-$	$Cl^- + NH_3 + OH^-$	$NH_2Cl + 2e^- + H_2O$	− 0.21	7.2	− 0.27

[a] Standard electrode potentials used: $ClO_2 + 5e + 4H^+ \leftrightarrow Cl^- + 2H_2O$, $E_o = 1.48V$; $I_2 + 2e \leftrightarrow 2I^-$, $E_o = 0.54V$; $HOCl + H^+ + 22e \leftrightarrow Cl^- + H_2O$, $E_o = 1.49V$; $OCl^- + H_2O + 2e \leftrightarrow Cl^- + H_2O$, $E_o = 0.9V$; $NH_2Cl + 2e + H_2O \leftrightarrow Cl^- + NH_3 + OH^-$, $E_o = 0.75V$. Computed values are applicable only to solutions of the redox pair in pure aqueous medium.

[b] $K_{eq} = [I_2]/[I^-]^2$ computed from Nernst equation.

[c] Computed using $[Ox] = 1 \times 10^{-4}$ M, $[I^-]_{gastric\ juice} \cong 1 \times 10^{-6}$ M; $EMF = \{E_o(Ox) + (0.0591/n) \log[Ox]\}\ \{E_o(Red) + (0.0591/n) \log [Red]\}$.

of disinfectants and that iodinated compounds are absorbed (Table 2). The logical conclusion from these observations is that under the oxidative influence of disinfectants, *in vivo* formation and absorption of a numerous diverse iodinated substances must occur. Most data in the literature relate only to *in vivo* organification of iodine under conditions mediated by cellular peroxidases, such as the broadly recognized iodination of thyroglobulin during thyroxine synthesis, or the iodination of arachidonic acid by myeloperoxidase of polymorphonuclear leukocytes (*14*).

Apart from the biological activity of thyromimetic and inhibitory analogs of thyroxine, or the metabolic fate and renal toxicity of radiocontrast preparations, virtually nothing is known about the toxicity of iodinated natural products. Feeding iodinated casein to test animals has been shown to accelerate the development of Vitamin B-12 and folate deficiency; however, no explanation for this effect was offered (*13*).

Currently, we hypothesize that some as-yet unknown iodinated molecule forming in trace quantities in the alimentary tract is responsible for the thyroid inhibition seen during ClO_2 exposure. It is anticipated that such compounds form *in vivo* in very small amounts even at 100 ppm ClO_2 concentration; therefore, they must possess extraordinary biological activity.

Furthermore, since the iodine substituent in organic molecules tends to be a reactive moiety prone to undergoing replacement reactions or carbocation formation, we postulate that iodinated compounds have pronounced genotoxic and carcinogenic activity. The *in vivo* formation, molecular structures, and biological activity of such compounds are under investigation.

This document has been subjected to EPA review and approved for publication. Mention of trade names or commercial products does not constitute endorsement or recommendation for use. The typing of the manuscript by Patricia Underwood is greatly appreciated.

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